Targeting cellular dysfunction with green tea polyphenols to treat the underlying basis of the metabolic complications of obesity

A thesis submitted for the Degree of Doctor of Philosophy

by

Weiguo Lao

School of Life Sciences

University of Technology Sydney

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Certificate of Original Authorship

I, Wei Guo Lao declare that this thesis, is submitted in fulfilment of the requirements for the award of Degree of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Abstract

Obesity is a metabolic disease with high risk of severe complications, including type 2 diabetes, cardiovascular disease, and cancer conditions. To develop natural approaches to obesity-associated metabolic complications a series of *in vitro* studies were conducted to evaluate effects of green tea polyphenols (GTP) on cellular dysfunction related to the development of obesity and its complications.

Increased differentiation of adipocytes from preadipocytes contributes to fat mass expansion and leads to obesity and type 2 diabetes. The effects of GTP on fat cell differentiation and lipids accumulation were investigated in Chapter 3 using 3T3-L1 preadipocytes. The results demonstrated that GTP suppressed the differentiation of 3T3-L1 preadipocytes into mature adipocytes through down-regulating adipogenic regulators of PPARγ, C/EBPα, and SREBP-1c at gene expression and post-transcriptional level. The findings from this study suggest that GTP can combat unnecessary adipogenesis at the cellular level to prevent obesity and subsequently reduce the risk of type 2 diabetes and cardiovascular disease.

Accumulating evidence demonstrated an increase of adipogenesis accompanied by osteoporosis in obese populations. Adipocytes and osteoblasts originate from a common ancestor, pluripotent mesenchymal stem cells. Based on the anti-adipogenic effect of GTP, the study in Chapter 4 investigated whether GTP possesses the ability to limit human adipose tissue-derived stem cells (hADSCs) differentiation to adipogenic lineage and concomitantly enhance osteogenesis. The study utilising hADSCs and PPARγ agonist identified PPARγ and Runx2 as essential regulators involved in adipogenesis and osteogenesis, respectively. GTP treatment inhibited ADSCs differentiation to mature adipocytes and promoted osteogenesis through suppressing the PPARγ-induced adipogenesis and upregulating Runx2-Bmp2

mediated osteogenic pathway. Therefore, the enhancement of osteogenesis with preventing adipogenesis with GTP may provide a rational approach towards developing GTP as multifaceted therapeutic goods for the intervention of obesity-associated osteoporosis.

Polycystic Ovarian Syndrome (PCOS) is a complex of the metabolic and reproductive disorder. Obesity associated with insulin resistance plays an important role in excessive androgenesis by ovarian theca cells. Based on our observations that GTP is capable of enhancing insulin-mediated glucose and lipid metabolism with anti-obese effect study in Chapter 5 investigated whether GTP would be able to alternate ovarian cell dysfunction led abnormal steroidogenesis using dexamethasone-induced hyperandrogenism in primary thecal cells from female mice. GTP treatment inhibited over-secretion of testosterone in theca cells through downregulation of the expression of the cytochrome P450 17a-hydroxylase (CYP17A1) additionally reduced CYP11A1 expression at the high concentration (25 μg/mL). These findings provide scientific evidence to support the use of GTP for ovarian dysfunction leading to hyperandrogenism in PCOS.

DNA methylation is essential for healthy development and is also involved in aging and carcinogenesis. The recent study using genome-wide DNA methylation analysis has identified hypermethylation as a potential epigenetic change of obesity-related cancer. Based on the antiadipogenic effect of GTP during ADSCs differentiation, the study in Chapter 6 evaluated the effects of GTP on DNA methylation in deferent passages of ADSCs. This study discovered GTP enhanced DNA methyltransferase 1 (biomarker of DNA methylation) expression without changing MYC expression at early stage of ADSCs passages and downregulated DNMT1 expression at passage 12 of ADSCs culture. These findings suggest GTP possesses dual regulatory effects on DNA methylation during ADSCs expansion with genomic stability, which highlights the potential use of this naturally occurring compound in

ADCSs expansion for regenerative medicine without carcinogenic risk.

In summary, *in vitro* studies conducted in this thesis have demonstrated beneficial effects of and molecular mechanisms of GTP on obesity and obesity-associated osteoporosis and PCOS. To gain deeper insight into therapeutic application of GTP for obesity and its metabolic complications, the animal studies and clinical trials are warranted in the future study.

Publications and communications

Refereed publications arising from this thesis

Lao WG., Jin X., Tan Y., Xiao L., Padula M. P., Bishop D.P., Reedy B., Ong M., Kamal M.A. and Qu X., Characterisation of Bone Beneficial Components from Australian Wallaby Bone, Medicines 2016, 3(3), 23; doi:10.3390/medicines3030023

Lao WG., Tan Y., Jin X., Xiao L., Kim JK. and Qu X., Comparison of Cytotoxicity and the Anti-Adipogenic Effect of Green Tea Polyphenols with Epigallocatechin-3-Gallate in 3T3-L1 Preadipocytes, Am. J. Chin. Med. 43, 1177 (2015). https://doi.org/10.1142/S0192415X15500676

Tan Y., Jin X., Lao WG., Kim JK., Xiao L. and Qu X., Anti-Resistin RNA Oligonucleotide Ameliorates Diet-Induced Non-Alcoholic Fatty Liver Disease in Mice through Attenuating Proinflammatory Cytokines, BioMed Research International, Volume 2015 (2015), Article ID 414860, 13 pages

Tan Y., Lao WG., Xiao L., Wang ZZ., Xiao W., Kamal M.A., Seale J.P. and Qu X., Managing the Combination of Nonalcoholic Fatty Liver Disease and Metabolic Syndrome with Chinese Herbal Extracts in High-Fat-Diet Fed Rats, Evidence-Based Complementary and Alternative Medicine, Volume 2013 (2013), Article ID 306738, 10 pages, http://dx.doi.org/10.1155/2013/306738

Xiao L., Lao WG., Tan Y. and Qu X., *In vitro* Investigation of Anti-diabetic Effect of Taxus cuspidate Extracts by Ultrasound Assisted Method. Am.J Chin Med. 2012, 40:1-11.

Papers presented at scientific conferences

Lao WG., Tan Y., Jin X., Xiao L., Kim JK. and Qu X., Green tea polyphenols inhibit adipogenesis in 3T3-L1 preadipocytes through regulating expressions of PPARγ, C/EBPα and SREBP-1c, New Horizons 2014 Conference, The Kolling Building, RNSH, Sydney, Australia, 17th - 19th November 2014, NH2014-8745

Lao WG., Ong M., Jin X. and Qu X., The effects of Green Tea Polyphenols on ovarian steroidogenesis in ovarian cells, The New Horizon 2016 Conference,33rd Combined Health Science Conference, City - Haymarket, CB08 Dr Chau Chak Wing Building, Building 8, UTS, Sydney, Australia, 21-22 November, 2016, NH2016-105317

Lao WG., Ong M., Jin X., Santos J. and Qu X., Potential therapeutic effects of green tea polyphenols on obesity associated osteoporosis, The Australian Diabetes Society (ADS) and the Australian Diabetes Educators Association (ADEA) Annual Scientific Meeting, Perth Convention & Exhibition Centre, Perth, Western Australia, 30th August-1st September 2017, ADAD-145478

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5mC	5-methylcytosine
AMM	adipocyte maintenance medium
ABS	Australian Bureau of Statistics
ADA	American Diabetes Association
ADSCs	Adipose-derived stem cells
ALP	Alkaline phosphatase
AS	Atherosclerosis
BFR	The Body Fat Ratio
BGP	Osteocalcin
BMD	Bone mineral density
BMI	Body Mass Index
Bmp2	Bone morphogenetic protein 2
NBS	Bovine Calf Serum
CVC	Cardiovascular complications
CVD	Cardiovascular Disease
C/EBP	CCAAT/enhancer-binding protein-a
CHD	Coronary heart disease
CYP11A	Cytochrome P450 family 11 subfamily A
CYP17A	Cytochrome P450 family 17 subfamily A
DEX	Dexamethasone

DMEM	Dulbecco's Modified Eagle Medium
DNMT	DNA methyl-transferase
EGCG	(−)-epigallocatechin-3-gallate
EM	Expansion Medium
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFAs	Free fatty acids
GLUT-4	Glucose transporter 4
GT	Green tea
GTE	Green tea extract
GTP	Green tea polyphenol
HDACs	Histone deacetylase
IBMX	Isobutyl-1-methyl-xanthine
IDF	International Diabetes Federation
IOF	International Osteoporosis Foundation
IR	Insulin resistance
IRS-1	Insulin receptor 1
IL-6	Interleukin- 6
LC-MS	liquid chromatography-mass spectrometry
MHO	Metabolically healthy obesity
MS	Metabolic Syndrome
NAFLD	Non-alcoholic fatty liver disease
NIDDM	Noninsulin-dependent diabetes mellitus

NWO	Normal weight obesity
OECD	Organization for Economic Co-operation and Development
PAI-1	Plasminogen activator inhibitor 1
PCOS	Polycystic ovary syndrome
PPARγ	Peroxisome proliferator-activated receptor γ
PPRE	PPARγ response element
PPC	Polyphenols and L-carnitine
qRT-PCR	Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction
RunX2	Runt-related transcription factor 2
RXR	Retinoid X receptors
SAS	Sleep apnoea syndrome
SAM	S-adenosylmethionine
SREBP-1c	Sterol regulatory element-binding protein 1c
TGF-β1	Transforming growth factor beta 1
TNFα	Tumor necrosis factor
TZD	Thiazolidinedione compounds
T2D	Type 2 diabetes
WHF	World Heart Federation
WHR	Waist-to-hip ratio
WHO	World Health Organization
TCM	Traditional Chinese medicine